Analysis of the major components of root exudates released from several economic forest tree using GC-MS

SUN Hao-yuan, WANG Yu-zhu*, YANG Li

Institute of Forestry and Pomology, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100093, P. R. China

Abstract: In 2000 and 2001, 1-year-old seedlings of 7 economic tree species including chestnut, apricot, persimmon, peach, walnut, pear and apple were cultured in garden pots that had a diameter of 40 cm and were filled with clean sand. The major components of exudates released from their roots were isolated and analysed by GC-MS. Totally 200 kinds of organic chemicals were isolated, of which 3 kinds i.e. naphthalene, dimethylbenzene and dibutyl phthalate were principally controlled pollutants according to "Blacklist of Principal Environment Pollutants in China" and the standard of U.S. Environmental Protection Agency (EPA). The research result provided theoretical evidence for selecting low-pollution economic forest crops in the water source protection area in Miyun Reservoir.

Keywords: Economic forest tree; Root exudates; Components

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Introduction

The improved environment ought to owe mostly to vegetation, especially the trees. However, it was reported recently that the volatile of some plant species can add to air pollution. Over 1400 tree species were compiled and rated for their various pollution impacts and pollen production in a study presented to the California Environmental Protection Agency's Air Resources Board. Besides the volatile emissions may increase air pollution, how about the relation between trees' root exudates and the soil environment? Root exudates from root of living plants show that a number of organic compounds are released into the soil. Studies on the allelopathic effect of root exudates were often stated in literature (Yamamoto *et al.* 1999; Kato 2001; Cao 1997; Yu 1999), but its negative effect on environment, particularly on the water resource, has rarely been researched. Therefore, these research aspects, such as the relatinship between root exudates and environmental pollution, would be the focus of future study (Mu 1996).

As the most important drinking water resource, water quality in Mi-Yun Reservoir directly impacts upon the public health in Beijing. It is true that the afforestation of economic forest trees in this region not only can improve the ecological condition but also increase the planters' income. On the other hand, the economic forest tree with large areas planting in the upriver region, which cover area is 1 487.1 km^2, may pollute Mi-Yun Reservoir, because of the un-neglectable amount of root exudates. In this paper, 7 kinds of economic forestry tree including chestnut, apricot, persimmon, peach, walnut, pear and apple were cultured in pot and the major components of root exudates released from their roots were isolated and analysed by GC-MS.

Materials and methods

Materials

In 2000 and 2001, 1-year-old seedlings of 7 economic forest tree species including chestnut, apricot, persimmon, peach, walnut, pear and apple were cultured in garden pots that had a diameter of 40 cm and were filled with clean sand. The tested plants all grew well in sameness condition. This trial repeated 3 times. Controlled pots with no seedlings were also filled with clean sand.

Methods

Routine treatment with the seedlings: Uniform treatments were performed on each seedling in a year-round period. After irrigation, water should not drip from the bottom ventage. Distilled water and Tukey Medium's macro-element solution (1 liter contains KNO_3 136 mg, $Ca(H_2PO_4)_2$ 170 mg, $MgSO_4$ 170 mg, $CaSO_4$ 170 mg, KCl 680 mg, $FePO_4{\cdot}2H_2O$ 170 mg) were used in turn to maintain the plants' well growing. In rainy season, the tested pots were covered with plastic to prevent them from being poured by rain.

Collection of the root exudates: Firstly, potted plants together with the containers were soaked in certain quantity of water for over one night. Secondly, they were leached for three times. Subsequently, the leaching water was collected and was filtered by filter paper, then, flew through XAD-2 resin column at a speed of 15-20 mL/min. Finally, the packed samples were ready to be detected.

Elution and purification: After having removed the water remained in resin columns by vacuum pump, we soaked

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Biography: SUN Hao-yuan (1972-), Ph. Doctor, Assistant Researcher Fellow in Institute of Forestry and Pomology, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100093, P. R. China.

*-Corresponding author (E-mail: lqzwyz@public.edu.cn).

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resin columns for 10 min (3 times repeat) with the extraction solvent hexane, such as acetone (85:15) and dichloromethane. The eluant was dehydrated by anhydrous Na_2SO_4, and then flew slowly through silica gel column in order that macromolecular organic chemicals, such as humic acid and so on, could be removed. Finally, the eluant was concentrated into 1 mL in the K-D concentrator. Before detection, the sample was fully dissolved by given solvent.

Parameters for GC-MS detection: HP5890 II Gas Chromatogram and 5791 Series Mass Spectrograph were applied in analyzing the root exudates. Temperature at the scoop was 240 °C. Column temperature at 50 °C lasted for 2 min, rose to 100 °C (lasted for 5min) at a speed of 5 °C /min, rose to 150 °C (lasted for 5 min) at a speed of 3 °C /min, rose to 250 °C (lasted for 10 min) at a speed of 10 °C/min, then GC-MS detection started. The flexible quartz capillary had an inner diameter of 0.32 mm and was 60 m in length. Using helium as carrier gas, 1μL sample was charged in each test. Ion source was 160 and the matters with molecular weight between 10.00 and 650.00 were scanned.

Data analysis: In the result of GC-MS analysis, components with more than 85% confidence rate were regarded as undoubtedly existed in the root exudates. The components that appeared in blank tests were not recorded in the final result.

Results and analysis

Organic chemicals in the root exudates

There were over 200 organic chemicals being isolated, as Curl estimated (Curl 1986), (Table 1). Root exudates can be divided into two types: one is general type that is common in most plants; the other is special, i.e. the components that are produced by certain plant species or under certain conditions (Tu *et al.* 2000). It was shown that different species had similar components as well as different ones (Table 1). Moreover, difference of components also occurred in the same species that were in different years. Some components existed in the form of isomer or homologue, others were completely different, because the plant root exudates were influenced by many factors such as plant genotype (Curl 1986), age (Kaverzina 1981), nutrition status (Tu *et al.* 2000) and so on.

Table 1. Major components of root exudates identified by GC-MS in different years

Tree species	Major components of root exudates	
	2000	2001
Chestnut	1,2,3-Trimethyl-Benzene; 1,2,4-Trimethyl-Benzene; Naphthalene*;1,2,3,4-Tetrahydro-Naphthalene; 1-Methylethenyl-Benzene; 1-Methyl-2-Cyclopropen-Benzene; 1-Methyl-1H-Indene	1,2-Benzisothiazol ;Bis(2-Ethylhexyl)Phthalate; Phthalic Acid,Diisooctyl Ester; 1,2-Benzenedicarboxylic Acid
Apricot	1,4-Diethyl-Benzene; 1,3-Diethyl-Benzene▲; P-Xylene*; 1,2-Dimethyl-Benzene*; 1,2,4-Trimethyl-Benzene; 1,2,3-Trimethyl-Benzene; 1,2,3,4-Tetrahydro-Naphthalene; Naphthalene*▲; 1-Methyl-1h-Indene; Cis,Trans,Cis-1-Isobuthyl-2,5-Dimethyl-Cyclohexane; Benzothiazole; Tridecane~Tricosane; Hexatricontane	Bis(2-Ethylhexyl)Phthalate; Naphthalene *▲
Persimmon	Bis(2-Ethylhexyl)Phthalate; 2,3-Dimethyl-1-Hexene; 1-Methyl-1H-Indene	Not Available
Peach	4-Ethenyl-1,2-Dimethyl-Benzene*; 2,3-Dihydro-5-Methyl-1H-Indene; Cis,Trans,Cis-1-Isobuthyl-2,5-Dimethyl-Cyclohexane	1,3-Dimethyl-Benzene*; Bis(2-Ethylhexyl)Phthalate;Benzaldehyde; 1-Methyl-Naphthalene
Walnut	4-Methyl-Benzaldehyde-Oxime; 2,3-Dimethyl-1-Hexane; 1-Methyl-2-(2-Propenyl)-Benzene; 2,3,5-Trimethyl-Hexane	1-Methyl-Naphthalene; Bis(2-Ethylhexyl)Phthalate
Pear	Cis,Trans,Cis-1-Isobuthyl-2,5-Dimethyl-Cyclohexane; Cis,Cis,Trans-1-Isobuthyl-2,5-Dimethyl-Cyclohexane	Pentadecyl; 1,3-Dimethyl-Benzene *; 1,2-Benzisothiazole; Tetradecane; Eicosane
Apple	1,2,4-Trimethyl-Benzene;Naphthalene*▲; Benzisothiazol;Dibutylphthalate▲; (1-Methyl-2-Cyclopropane)Benzene	Naphthalene*▲;Benzisothiazol; Bis(2-Ethylhexyl)-Benzendicarboxylic Acid

Notes: *----Principally controlled pollutants listed by State Environmental Protection Administration of China (SEPA); ▲----Principally controlled pollutants listed by U.S. Environmental Protection Agency (EPA).

Principally controlled pollutants in root exudates from different species

Compared with principally controlled pollutants prescribed by SEPA and EPA, organic pollutants do exist in the root exudates. Three components belong to principally controlled pollutants i.e. naphthalene, dimethylbenzene and dibutyl phthalate, some of others including trimethyl-benzene, tetrahydro-naphthalene, ethylbenzene, 4-methyl-benzaldehyde-oxime, 1-methyl-2-cyclopropen-benzene, benzaldehyde, 1-methyl-naphthalene and pentadecyl, etc. are also organic pollutant. In 2000 and 2001, naphthalene was isolated in root exudates from apple and apricot tree; dimethylbenzene from peach (Table1). Only in one year, dimethylbenzene was isolated in root exudates from chestnut and pear tree; p-xylene and dimethyl-benzene from apricot; dibutyl phthalate from apple (Table1). No principally controlled pollutant was detected in root exudates from walnut tree. Because of the difference in root exudates from different tree species, it is necessary to select low-pollution tree species if large scale planting would be implemented in the area of water source protection.

Field samples analysis

In order to confirm the result of potting test and its feasibility in studying the root exudates, we selected 1-year-old walnut seedlings, whose root together with the soil in the

rhizosphere was detected as field sample, at Miyun County. The result of GC analysis showed that field sample's components were very complicated and the abundance was very high (Fig. 1). The chemicals in the soil would interfere with the detection. Consequently, adopting container culture could reduce the effect of organic chemicals in soil and increase reliability of the result (Table 2).

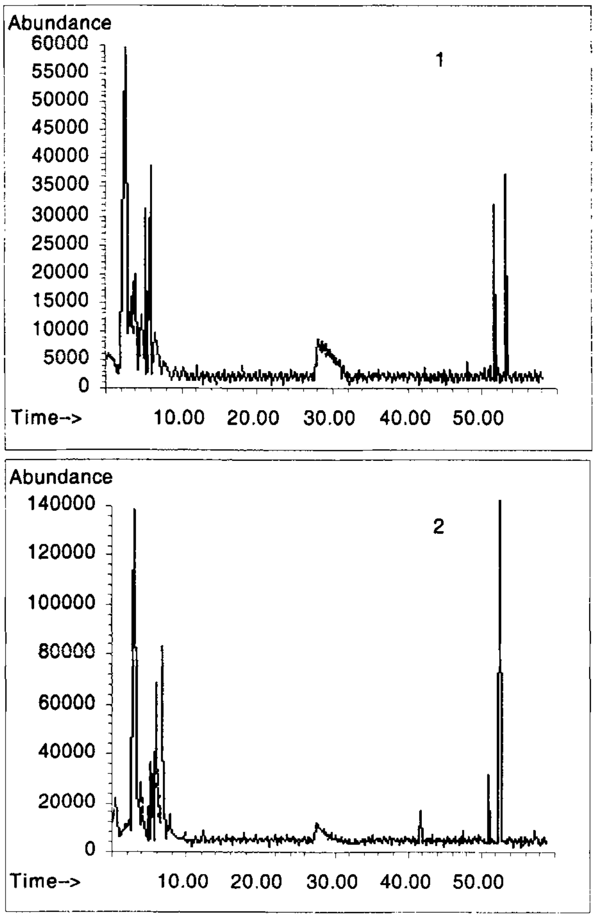

Fig.1 Results of GC analysis of field samples
(1. Walnut; 2. Soil sample)

Just like the pot culture test, 4-methyl-benzene- formaldehyde oxime and 2,3,5-trimethyl-hexane were isolated in the field sample. This proved that container culture is feasible in study of root exudates. As to the other components detected only in field sample, it indicated that conditions in pot culture was not equal to natural conditions under which plants were influenced by various factors such as water stress, soil conditions and microorganism in rhizosphere and so forth. As a result, there appeared different quantity or different kinds of root exudates. This is similar to the studies of Prikry (Prikry 1980) and Sharp (Sharp 1994).

Discussion

GC-MS is an effective method that has been widely used in detecting organic chemicals. However, it is unable to identify those nonvolatile and heat-stable matters. For instance, some ropy and butyraceous substances that have high polarity and high MW, are likely to be the main components in increasing water pollution, but they are difficult to be determined by GC-MS. In order to get a perfect and practical result, LG-MS should be applied in the future study.

Table 2. Major components of root exudates of field sample identified by GC-MS detection

Tree species	Major components of root exudates
Walnut	4-Methyl-Benzene-Formaldehyde Oxime; 2,3,5-Trimethyl-Hexane; 3-Ethyl-4-Methyl-1-Pentene; 1,2-Benzenedicarboxylic Acid; 1,2-Benzenedicarboxylic Acid, Buty

Although the quantities of the chemical pollutants in root exudates need to be further tested, some species planted in large scale is supposed to affect water quality through regional concentrations of organic pollutants and it is particularly important to use low secreting species. When carrying out an afforestation project in the large area of water source protection, we suggest that the content of root exudates from tree species should be systematically determined. Therefore, we can scientifically select the cleanest tree species based on the research results, at same time, protect public health and ecological resources through effective reduction of water pollutants while recognizing and considering effects on the economy.

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